390-Pos Board B159
The Assembly and Control of the XerCD-dif Recombination Machinery Studied at the Single Molecule Level
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Tyrosine recombinases are well known to catalyse site-specific DNA recombination in bacteria, archeae and eukaryotes. In bacteria, these recombinases are extensively used for programmed integration, excision and inversion of DNA segments. XerC and XerD form together a highly conserved tyrosine recombinase devoted to recombine dif sites, located in the terminal domain of circular bacterial chromosome. XerCD-dif recombination resolves chromosome dimers to monomers before segregation and is thus required for the faithful segregation of sister chromosomes in daughter cells. To do so, its activity is precisely tuned and controlled during the bacterial cell cycle. In E. coli, XerCD/dif activity is controlled at two levels: i) the direction of the recombination reaction (from dimer to monomer and not the inverse) and ii) the timing of the reaction (coupled to septation). Both controls necessitate the cell division protein FtsK. The way this septal DNA translocase acts on XerCD/dif recombination is not completely understood but involves the control of the assembly of the nucleoproteic complex where recombination takes place.
To understand the XerCD-dif recombination and its FtsK-mediated control, we study the assembly of the recombination complexes on single DNA molecules. We will present the Tethered Particule Motion experimental setup we are using as well as our findings.

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AZT Drug Resistance Conferred by a Dipeptide Insertion in the Fingers of HIV-1 Reverse Transcriptase Involves Multiple Mechanisms
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HIV reverse transcriptase (RT) mutants containing a dipeptide insertion between codons 69 and 70 of HIV-1 RT (the ''69 insertion complex'') are resistant to all nucleoside/nucleotide analog reverse transcriptase inhibitors (nRTIs) currently approved by the US FDA. Pre-steady-state kinetic studies and global data analysis were performed to determine the mechanistic basis for the drug resistance against AZT by RTs containing the 69 insertion complex. We first measured kinetic constants governing nucleotide binding and incorporation for TTP and AZTTP by two chimeric RTs: the T69SSS and the 69 insertion complex. We showed that TTP binds more weakly to both mutants than AZTTP. However, this weaker ground-state binding is offset by the much more favorable substrate-induced isomerization step while rates of chemistry for TTP and AZTTP by each mutant are comparable. The results indicate that the fingers insertion only modestly improves discrimination against AZT. Consequently, it has been widely accepted that resistance arises predominantly by increasing rates of ATP-dependent excision of the chain terminator. However, data from our primer rescue and extension assays showed that that is not the case: the TAMs mutant increased the excision efficiency by ~5-fold while the introduction of fingers insertion into the TAMs background actually decreased the excision efficiency to only ~2 fold. On the other hand, the fingers insertion did interfere with the binding of the next correct nucleotide (dCTP), making its binding weaker by ~1.5 fold. This change, however, will not affect the excision efficiency at the physiological nucleotide concentration due to the weak binding of dCTP to the 3'-end of AZT terminated primer. Thus, the discrimination and excision mechanisms cooperate together at multiple transcription sites, which conferred the clinically observed high level of nucleotide resistance.

392-Pos Board B161
Visualization of UV-Induced Damages on Single DNA Molecules
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UV radiation-damaged DNA such as DSB and SSB are visualized at the level of single DNA molecules using light microscopy. Single molecule observations provide a map of DNA radiation-mediated breakage, revealing sequence-dependent DNA damage. These findings suggest that essential genes for survival have evolved to be tolerable to short wavelength UV radiation.

393-Pos Board B162
Understanding the Kinetic Mechanism of MutS during Mismatch Recognition and Initiation of Repair
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DNA mismatch repair (MMR) is essential for correcting errors generated in DNA during replication. Abnormal expression or function(s) of MMR proteins give rise to a mutator phenotype, increasing cancer susceptibility. MMR initiates when MutS recognizes a mismatch and binds it with high affinity. MutS then binds ATP and forms a sliding clamp to signal initiation of downstream repair steps. The DNA-binding and ATPase activities of MutS are under active investigation, in particular the events involved in transition between recognition of errors and initiation of repair. We are utilizing stopped-flow analysis of fluorescently labeled protein as well as DNA to determine the kinetic mechanism of Thermus aquaticus MutS. Our results indicate that MutS rapidly interacts with mismatches to form an initial weak complex (KD1 = 2 μM), followed by conformational changes in both DNA and MutS to form a high affinity MutS-DNA complex with the DNA kinked at the mismatch site (kconf ~ 20 s-1 and KD2 = 15 nM). In absence of mismatched DNA, MutS rapidly switches between two conformations in response to ATP binding and hydrolysis. ATP hydrolysis is suppressed in mismatch-bound MutS, and our data suggest that after ATP binding (kON = 0.2 μM-1s-1), the MutS-DNA complex undergoes at least two conformational changes (kobs1 ~ 3 s-1 and kobs2 ~ 0.3s-1) possibly related to DNA unbending and formation of the MutS sliding clamp, respectively. By monitoring the reaction via fluorescent reporters on both DNA and protein, we are developing a comprehensive view of MutS actions on DNA and gaining novel mechanistic insights into the initiation of mismatch repair.

394-Pos Board B163
Global and Local Conformational Studies of Mismatched Duplex DNA Upon Msh2-Msh6 Binding by Steady-State and Time-Resolved Fluorescence
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The DNA mismatch repair (MMR) system guards the integrity of genetic material by scanning and correcting errors in a post-replicative manner and thus improves the fidelity of DNA replication by several orders of magnitude. In eukaryotic cells, the initiation of MMR is achieved by recognition of biosynthetic errors by Msh proteins (MutS homologs). Single base mismatches and small insertion/deletion loops (IDL) are recognized by the Msh2-Msh6 heterodimer followed by recruitment of MutL homologs (Mlh/Pms) and this ATP-dependent ternary complex further activates downstream MMR events. The exact mechanism by which Msh2-Msh6 distinguishes a mismatched base pair precisely from a large excess of canonical Watson-Crick base pairs in an efficient manner is still unknown. In this study we explore the specificity of Msh2-Msh6 binding that enables discrimination between different mismatches through measurement of binding affinity and protein-induced bending using fluorescence anisotropy, gel mobility shift assays and Förster Resonance Energy Transfer (FRET). These measurements yield the following order of binding affinity: G:T > +T > G:A > G:C and are in good agreement with previous results. The FRET efficiency of free and Msh2-Msh6-bound mismatched duplex DNA suggests that Msh2-Msh6 bends both +T and G:T duplex DNA relative to G:C homoduplex DNA, which appears to remain relatively straight in the bound form. We have also monitored local DNA base pair dynamics with time-resolved fluorescence intensity and anisotropy spectroscopy measurements. By using a fluorescent nucleoside analog, 6-methylisoxanthopterin incorporated into mismatched DNA, we can explicitly investigate single base pair dynamics. These experiments reveal that protein binding stabilizes the probe placed at the mismatch or located adjacent to the unpaired thymine. This stabilization is relatively local to the mismatch site and is not propagated down the helix.

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POT1/TPP1 and Telomeric G-Quadruplexes Synergistically Block RPA's Access to Telomeres
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Human telomeres terminate with a G-overhang, which can be misrecognized as a DNA damage site by RPA. In cells, POT1/TPP1 protects telomeres against RPA binding. The G-overhang folds into different G-quadruplex (GQ) conformations. The role of GQ formation in protection of telomeric overhangs remains unclear. Using single-molecule FRET, we show that the ability of POT1/TPP1 to compete against RPA binding to telomeres is significantly enhanced by GQ formation. In the absence of POT1, RPA efficiently unfolds single-stranded telomeric GQs and binds to the unfolded DNA. POT1 and POT1/TPP1 stably load on to a 3'-TTAG sequence adjacent to a folded GQ. As a result of this loading two possible pathways emerge: 1) A fraction of GQs are unfolded at a level that depends on their folding conformation. The unfolded GQs are bound by POT1 or POT1/TPP1 which provides protection against RPA binding. 2) The GQs that remain folded after POT1 or POT1/TPP1 loading are greatly stabilized against RPA mediated unfolding, which